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PERCENTAGE OF ANTI-CD4 MONOCLONAL ANTIBODY–COATED LYMPHOCYTES IN THE RHEUMATOID JOINT IS ASSOCIATED WITH CLINICAL IMPROVEMENT

Implications for the Development of Immunotherapeutic Dosing Regimens

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Objective. **We assessed the effect of a daily dosing schedule of the chimeric anti-CD4 monoclonal antibody (MAb), cM-T412, in rheumatoid arthritis (RA) patients, and compared lymphocyte changes in the peripheral blood (PB) and synovial fluid (SF) of these patients.**

Methods. **Twelve patients received 50 mg/day of cM-T412 for 5 days, followed by a maintenance treatment of 50 mg/week for 5 weeks (6 patients), or a retreatment course of 50 mg/day for 5 days after 5 weeks (6 patients). Paired PB and SF samples were obtained during treatment for analysis.**

Results. **Changes in lymphocyte count and coating with the MAb in PB did not reflect changes in the SF. After 5 daily treatments, the percentage of cM-T412–coated CD4+ lymphocytes in SF correlated with the degree of clinical improvement seen in patients at 2 weeks after the initiation of therapy ($r = 0.75$, $P < 0.05$).**

Conclusion. **These results demonstrate the importance of antibody dosage and treatment regimen in determining clinical benefit. Our findings suggest that the percentage of cM-T412–coated CD4+ lymphocytes in SF may be a predictor of clinical outcome.**

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The chimeric anti-CD4 monoclonal antibody (MAb), cM-T412 (Centocor), has been tested as a treatment for rheumatoid arthritis (RA) in a number of open clinical trials (1–3). Studies using daily doses of cM-T412 have suggested that it was effective, although clinical response was variable and did not correlate with the degree of peripheral blood (PB) CD4 lymphopenia (1,3). We propose that there are at least 2 possible explanations for the variable clinical efficacy seen in these studies: first, the antibody dosage and treatment regimen may be important in determining clinical outcome; and, second, changes in PB CD4+ T lymphocytes after treatment with cM-T412 may not reflect changes in the synovial fluid (SF). We therefore undertook the present study in order to investigate these possibilities.

PATIENTS AND METHODS

Patients and treatment regimens. Twelve patients with RA as defined by the American College of Rheumatology (formerly, the American Rheumatism Association) 1987 revised criteria (4) were recruited from rheumatology outpatient clinics. They had active disease as defined by the presence of ≥4 swollen joints (SJ) and the presence of at least 2 of the following criteria: 1) erythrocyte sedimentation rate (ESR) ≥30 mm/hour; 2) early morning stiffness (EMS) ≥45 minutes; and 3) ≥9 tender joints (TJ). Disease-modifying antirheumatic drugs (DMARDs) were stopped 4 weeks prior to initiation of the study treatment. Stable doses of concurrent oral steroid equivalent to ≤10 mg/day of prednisolone were allowed. Patients were randomized into 2 treatment groups: group A (maintenance) or group B (retreatment). At week 0, all patients received 50 mg of cM-T412 daily, intravenously, for 5 days as an induction course. Group A (6 patients) received 50 mg of cM-T412 weekly for 5 consecutive weeks, from week 2 to week 6, while group B (6 patients) had no further MAb treatment until week 6, when they received 50 mg of cM-T412 daily for

Table 1. Clinical responses (mean ± SD) in group A and group B patients

Parameter, group	Week 0	1	2	4	6	8	14
Tender joint count							
Group A	73 ± 24	29.3 ± 5.4	36.7 ± 11.5	38.5 ± 17	38.8 ± 18	38 ± 18.6	21.3 ± 30.2
Group B	43 ± 14	21.5 ± 11	21 ± 15.8	23.2 ± 18	33.2 ± 25.5	25.3 ± 18.6	21.3 ± 20.2
Swollen joint count							
Group A	20 ± 6	8.2 ± 2.7	10.8 ± 3.6	9.5 ± 7.5	10.8 ± 4.4	10.5 ± 5.3	10.8 ± 7.5
Group B	15.7 ± 5.9	7.7 ± 3.6	7.3 ± 6.5	9.8 ± 7.5	10.2 ± 7.4	7.8 ± 5.4	9.3 ± 7.8
Early morning stiffness (minutes)							
Group A	510 ± 169	165 ± 221	265 ± 263	205 ± 203	273 ± 265	255 ± 275	365 ± 266
Group B	390 ± 233	170 ± 229	243 ± 284	252 ± 293	263 ± 283	218 ± 297	292 ± 283
Visual analog scale of pain (cm)							
Group A	8.2 ± 1.5	5.1 ± 2.9	6.9 ± 2.2	5.9 ± 2.8	6.9 ± 2.4	6.8 ± 2.9	5.4 ± 4.2
Group B	7 ± 1.7	3.8 ± 2.9	4.3 ± 2.5	4.9 ± 3.4	5.2 ± 3.6	4.7 ± 3.5	5.4 ± 4.2

5 days. In group A, if the PB CD4 count dropped below 50×10^6/liter, cM-T412 was replaced by a placebo infusion.

Clinical and immunologic assessments. RA disease activity was measured by the visual analog scale for pain, EMS, grip strength, TJ score (maximum 195), SJ count (maximum 62), patient's and physician's global assessment of disease activity (graded from 1 to 5), ESR, and levels of C-reactive protein (CRP). These measures were performed weekly from weeks 0 to 10, and at weeks 14 and 16.

PB mononuclear cell subsets were measured by immunofluorescence (IF) and analyzed by flow cytometry, using the MAb Leu-4–fluorescein isothiocyanate (FITC), Leu-4–phycoerythrin (PE), Leu-3a–PE, Leu-2a–PE, Leu-18–FITC, Leu-11a–FITC, Leu-16–FITC, Leu-M3–FITC (Becton Dickinson, Palo Alto, CA), and UCHL1–FITC (Dako, Santa Barbara, CA). Binding of cM-T412 to T cells was performed on whole blood samples, as previously described (2), using IF with Leu-4–PE and rabbit anti-human Ig Fc MAb (Dako). In 6 patients, paired PB and SF samples were available for IF analysis. These were obtained before treatment and 1 hour after infusion on days 1 and 5. SF samples were diluted 5-fold with phosphate buffered saline, centrifuged to obtain cell pellets, and IF was performed as described above for the PB samples. Levels of SF cM-T412 were determined by enzyme-linked immunoassay.

Statistical analysis. Results were analyzed on an intention-to-treat basis. Percentage of clinical improvement was calculated for each assessment parameter as follows: the value at week 0 was subtracted from the value at each treatment time point, and the difference at each time point was then divided by the value before treatment and multiplied by 100%. The mean of the different assessment parameters was expressed as "mean percentage improvement" before statistical analysis was performed. Differences before and after treatment were analyzed by Wilcoxon rank sum test. Differences between groups A and B were analyzed by Mann-Whitney U test. Correlation between clinical improvement and the percentage of SF cM-T412–coated CD4+ lymphocytes was calculated by Spearman's rank correlation.

RESULTS

There were no statistically significant between-group differences in patient age (group A mean ± SD 55 ± 19 years, group B 56 ± 13 years), duration of RA (group A mean ± SD 11.4 ± 7 years, group B 10.5 ± 9 years), or number of failed DMARDs (group A mean ± SD 3.8 ± 1, group B 4.3 ± 1.8). All patients had erosive disease, and all but 1 were rheumatoid factor positive.

Eleven patients completed the study and the treatment was well tolerated. One patient in group A withdrew before the last infusion when she developed an urticarial skin rash, which remitted spontaneously. One patient experienced chills and rigor after the first infusion of cM-T412. This resolved spontaneously and did not recur on subsequent treatment. The patient received only 4 maintenance doses of 50 mg of cM-T412. The last dose was replaced by placebo because her PB CD4 count dropped below 50×10^6 cells/liter at week 6. One patient developed mild hypotension (blood pressure dropped from 110/70 mm Hg to 90/60 mm Hg) that lasted 30 minutes and recovered spontaneously after the patient lay in a supine position. It did not recur with further treatment. No infectious complications were seen.

Clinical efficacy. After the first treatment course, there was a statistically significant disease improvement of 50% in group A patients ($P < 0.05$) and 53% in group B patients ($P < 0.05$), at week 1. Details of clinical effects are shown in Table 1. In group A, 2 patients had >50% improvement at week 1, while the rest had >20% reduction in disease activity. In most cases, the disease began to relapse by week 2, but, with weekly therapy, there was more sustained disease improvement. By week 16, most patients' disease had relapsed to pretreatment levels, but 2 patients continued to have substantially improved disease. Group B patients responded similarly after

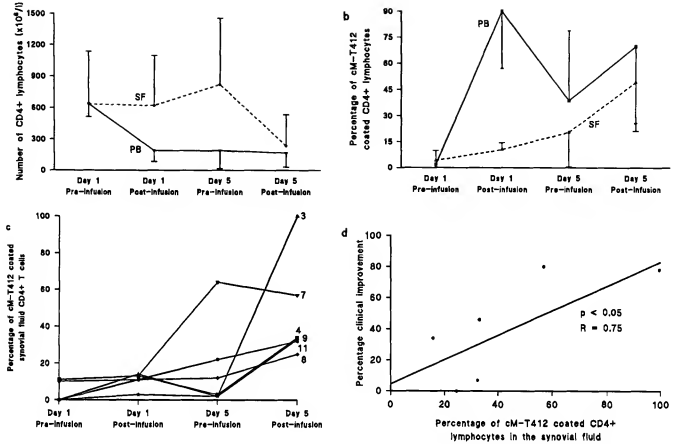

Figure 1. a, Number of CD4+ lymphocytes in the peripheral blood (PB) and synovial fluid (SF), pre- and posttreatment with cM-T412, on days 1 and 5. Values are the mean and SD. b, Percentage of cM-T412–coated lymphocytes in the PB and SF, pre- and posttreatment with cM-T412, on days 1 and 5. Values are the mean and SD. c, Percentage of synovial fluid CD4+ cells that were coated with cM-T412 concentration, pre- and posttreatment with cM-T412, on days 1 and 5, for individual patients. Lines are labeled with patient number. d, Correlation between the percentage of clinical improvement seen at week 1 and the percentage of cM-T412–coated lymphocytes in the SF, posttreatment, on day 5.

the initial treatment course: 3 patients achieved >50% and 2 patients had >20% disease improvement, while only 1 patient's disease failed to respond. The disease began to relapse after week 1 so that, by week 6, disease activity had returned to pretreatment levels for 4 patients. Only 2 patients had a sustained clinical improvement of >50%. After the second treatment course, 2 patients continued to have excellent improvement, but others only had transient disease amelioration. In all, disease improvement in 3 patients lasted more than 1 year. Similar to the findings of previous studies of cM-T412, no significant changes in ESR or CRP were seen (data not shown).

Lymphocyte subset analysis. After a single dose of 50 mg of cM-T412, there was a drastic reduction in the number of PB CD4+ lymphocytes, from a mean ± SD of 637 ± 123 × 10^6/liter to 190 ± 105 × 10^6/liter ($P < 0.001$; Figure 1a) in the 6 patients with paired PB and SF samples. In contrast, no statistically significant change was seen in the SF CD4+ lymphocyte number (from 635 ± 500 × 10^6/liter to 622 ± 477 × 10^6/liter). After 5 daily infusions, there was a reduction in the mean ± SD number of SF CD4+ cells to 237 ± 299 × 10^6/liter ($P < 0.05$). After a single dose of cM-T412, >90% of PB CD4+ lymphocytes were coated with cM-T412, compared with 11% in the SF (Figure 1b). After 5 doses of cM-T412, the percentage of cM-T412–coated SF CD4+ cells increased to 47%. The SF cM-T412 concentration after a single 50 mg dose was 6.5 ± 8.7 ng/ml and, after 5 daily doses of cM-T412, it was 143.3 ± 95.4 ng/ml, although there was a large variation among patients (Figure 1c). Interestingly, there was a statistically significant correlation ($r = 0.75$, $P < 0.05$) between the number of SF CD4 cells

coated with cM-T412 and the mean clinical improvement seen in each patient, at week 1 (Figure 1d). However, the correlation between the SF concentration of cM-T412 and clinical response was not statistically significant ($r = 0.37$, $P = 0.23$).

PB CD4+ lymphocyte number reflected the MAb dosage and treatment regimen. After the initial treatment course, there was a marked reduction in the PB CD4+ cell number (group A 731 $\times 10^6$/liter to 245 $\times 10^6$/liter, $P < 0.0001$; group B 664.5 $\times 10^6$/liter to 353 $\times 10^6$/liter, $P < 0.0001$). In group A, after weekly treatments, this steadily decreased to 149 $\times 10^6$/liter at week 8, and increased gradually to 218 $\times 10^6$/liter at week 16. In group B, the PB CD4+ cell number remained unchanged between weeks 2 and 6. The second treatment course reduced the PB CD4+ cell number to the same level (115 $\times 10^6$/liter) that was seen in group A patients at week 8. Thereafter, the 2 groups showed similar increases in CD4+ cell number, which depended on whether patients were taking concurrent steroids. At 24 weeks, 6 of 7 patients who were not taking steroids had PB CD4+ lymphocyte counts of >250 $\times 10^6$/liter, while the 5 patients taking oral prednisolone (dose range 5–7.5 mg/day) had a CD4 cell count below 250 $\times 10^6$/liter ($P = 0.02$ by χ^2 test).

DISCUSSION

Most patients who received cM-T412 showed transient disease improvement after the first treatment course, although 3 patients achieved prolonged disease improvement that lasted at least 12 months. However, their clinical response did not correlate with the degree of PB CD4 lymphopenia. Paired PB and SF sample data revealed that after a single 50-mg dose, cM-T412 coated PB but not SF CD4+ cells. It was only after 5 daily doses that sufficient cM-T412 reached the joint to coat SF CD4+ cells; coating in the SF was highly variable. This may be due to cM-T412 binding to CD4+ lymphocytes, monocytes, and reticuloendothelial cells. Only after 5 daily infusions is there sufficient cM-T412 available to enter the joint for binding to synovial CD4+ lymphocytes. The lack of correlation between cM-T412 concentration in the SF and clinical improvement may be due to cM-T412 binding to other CD4 targets in the joint, but not least to soluble CD4. The most interesting finding of this study is the correlation between the percentage of SF cM-T412–coated CD4+ lymphocytes and the clinical response. This suggests that cM-T412 may act by interfering with synovial CD4+ lymphocyte function. Notably, when almost all the CD4+ cells were coated, there was prolonged disease improvement that lasted up to 2 years. The number of patients studied was small; therefore, this finding will require confirmation in larger studies. However, it may be concluded that, in order to achieve significant disease improvement, the dose and the treatment regimen must deliver high concentrations of cM-T412 into the joints.

Some may object that this was an open study from which it would be difficult to draw reliable conclusions. We designed an open study because the correct dose and dosing regimen for cM-T412 were unknown. This is important since another placebo-controlled trial, using weekly dosages of 50 mg, showed no clinical benefit (2). Hence, existing studies may have negative results because of inadequate dosing and not necessarily due to lack of efficacy of the MAb itself.

The mechanism of the prolonged CD4 lymphopenia is unknown, but is dose related, and drug interactions may play an important role. Extremely prolonged CD4 lymphopenia occurred in patients treated with cM-T412 and methotrexate (3). None of our patients were taking DMARDs, and we have not seen such prolonged CD4 lymphopenia. However, the study patients who were receiving low-dose prednisolone had significantly longer lymphopenia than those not taking steroids. This may explain the drastic CD4 lymphopenia seen in an elderly patient taking both high-dose steroids and methotrexate; this patient developed pneumocystis infection (3). One major concern related to prolonged CD4 lymphopenia is the development of nonspecific immunosuppression and opportunistic infections. Our data showed that despite PB CD4 lymphopenia, a large number of CD4 cells are still present in the SF. If one assumes that CD4+ T cells at other extravascular sites are similarly untouched, then this may explain the absence of infectious complications seen in patients who have received cM-T412.

In summary, we have demonstrated clinical improvement after daily cM-T412 treatment in patients with refractory RA, and a high percentage of cM-T412–coated CD4+ lymphocytes in the joint may be associated with this therapeutic effect. Therefore, the ideal therapeutic regimen should aim to achieve high cM-T412 concentrations in the joint. Further studies are clearly needed in order to fully evaluate such a promising and exciting immunotherapy.

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